Exhibit 6

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Brain Activity and Clinical Outcomes in Adults With Depression Treated With Synchronized Transcranial Magnetic Stimulation: An Exploratory Study

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Background: Synchronized transcranial magnetic stimulation (sTMS) imparts low-amplitude magnetic stimulation matched to each patient's individual alpha frequency. It may act through entrainment of brain oscillations.

Objectives: To explore sTMS effects on neurophysiology with electroencephalography (EEG) in adults with major depressive disorder.

Methods: As an ancillary study to a clinical trial of sTMS, EEGs were recorded at baseline and at one and six weeks of treatment. Associations between EEG measures and clinical symptoms were examined.

Results: Absolute and relative power measures did not differ significantly between active and sham groups and did not change significantly over time. Changes occurring over six weeks in alpha current source density at anterior and central midline voxels were significantly correlated with changes in symptoms in subjects treated with active but not sham sTMS.

Conclusion: Neurophysiologic measures suggest that active but not sham sTMS engages brain targets, and that target engagement is related to treatment outcome.

Keywords: Current source, depression, EEG coherence, LORETA, synchronized TMS

Conflict of Interest: Dr. Cook discloses that UCLA has received research funding from NeoSync related to the analyses of this work and their NND-3001 and NND-3002 studies. In the past 36 months, he has received research funding from NIH as well; has been a consultant to Arctica Health, Cerêve, HeartCloud, NeuroDetect, and NIH (reviewer); he is editor of the Patient Management section of the American Psychiatric Association's *FOCUS* journal; his biomedical intellectual property is assigned to the Regents of the University of California; he has stock options in NeuroSigma and has served as Senior Vice President and Chief Medical Officer (on leave since 2016); he is employed by the University of California, Los Angeles and also has an appointment as a Staff Psychiatrist, Neuromodulation and Mood Disorders programs, Greater Los Angeles Veterans Administration Health System. Dr. Corlier reports that her intellectual property is assigned to the Regents of the University of California. Mr. Wilson has been a consultant to HeartCloud. Dr. Leuchter has received research support from Neuronetics, Breast Cancer Foundation, CHDI Foundation, and Neurosigma. He has served as a consultant to Ionis Pharmaceuticals, CHDI Foundation, and NeoSync, Inc. He serves as Chief Scientific Officer for Brain Biomarker Analytics LLC (BBA) Dr. Leuchter has stock options in NeoSync, Inc. and equity interest in BBA.

INTRODUCTION

Synchronized Transcranial Magnetic Stimulation (sTMS) is a new neuromodulation technique, examined in clinical trials in Major Depressive Disorder (MDD) (1,2). Repetitive TMS (rTMS) devices generally produce a pulsed magnetic field of >1T intensity by passing a rapidly-changing current through a coil at standard frequencies of stimulation (e.g., 10 or 18 Hz); in contrast, sTMS employs a sinusoidally changing magnetic field, generated by rotating a set of permanent magnets under microcomputer control (cf. Cook (3)). Rotational speed is set so that the magnetic field matches each individual's alpha frequency (IAF), measured by electroencephalography (EEG) prior to treatment. The rationale for this tuning includes patterns of frontal alpha activity associated with psychosis and treatment (cf. Jin (4)). Clinical studies of rTMS performed with pulsed stimulation at IAF have reported greater symptom improvement in both negative and positive symptoms of schizophrenia

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using alpha-guided rTMS than at other stimulation frequencies or with sham (4,5) suggesting tuning to IAF may offer therapeutic advantages. Unlike rTMS pulses which cause neuronal depolarization in brain tissue near the coil, the magnetic field of the sTMS device (NeoSync, Inc., Woburn, MA, USA) is several order of magnitude lower in amplitude and does not trigger action potentials.

It has been proposed that the mechanism of action of sTMS may involve entrainment of brain oscillatory activity (e.g., EEG signals) (6,7). We examined EEG data recorded from subjects from the double-blind, sham-controlled "NND-3001" study [NCT01370733 (2)] as an ancillary study to that trial at the UCLA site, to explore the relationships between brain electrical activity and clinical metrics during sTMS treatment and to generate hypotheses for future research, including EEG markers of target engagement which might clarify how responders and nonresponders may differ.

METHODS AND SUBJECTS

In the primary protocol for the NND-3001 study, medication-free adults with MDD were enrolled in a clinical trial that began with a six-week double-blind period of daily active or sham sTMS. Treatment sessions lasted 30 min, using the NeoSync EEG Synchronized TMS ("N.E.S.T.") device, the full details of which are described in the primary report of that study (2). Subjects reclined on a table while the N.E.S.T. device was placed in contact with the head, with its internal rotating magnets aligned along the midline, exposing frontal and central cortical regions to the stimulating field. Clinical symptoms, assessed with the self-rated inventory of depressive symptomatology (IDS), were considered here at baseline and last treatment session. All procedures were reviewed and approved by the IRB prior to execution of the study.

In this ancillary study, full-head 35 channel EEG recordings were performed in the maximally-awake, eyes-closed condition, with a Pz reference (ElectroCap, Inc., Eaton, OH, USA). Signals were recorded at a sample rate of 256 Hz, with a passband of 0.3–70 Hz, and a notch filter at 60 Hz. EEG preprocessing and artifact rejection was performed using the Brain Vision Analyzer software (Brain Products GmbH, Gilching, Germany) for removing movement, ocular, heart, and muscle artifacts. Between 20 and 30 sec of artifact free data were available for 16 individuals: ten randomized to active sTMS and six to sham.

Analyses were performed using MATLAB (R2017b, Mathworks, Inc.) along with EEGLAB toolbox (UCSD, https://sccn.ucsd.edu/eeglab), and LORETA-KEY (University of Zürich, http://www.uzh.ch/keyinst/loreta.htm) software packages. Absolute and relative EEG power was calculated using the classically-defined bands of delta (0.5–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), and beta (12–20 Hz). The frequency power spectrum was calculated using Welch's power spectral density estimate, using 2 sec long segments sampled at 256 Hz (frequency resolution of 0.5 Hz). Relative power values for each frequency band are expressed as the percentage of total power in the range 0.5–20 Hz. The width of the alpha spectral peak also was examined using the *Q*-factor metric (getQualityFactor function in MATLAB) for the FPz-Oz channel. Current source density (CSD) also was evaluated with low resolution brain electromagnetic tomography (LORETA-KEY). LORETA computes current density as a linear weighted sum of scalp electrical potentials. It does not assume a specific number of sources for solving the "inverse problem," but assumes that neighboring voxels are similarly active. Based on this assumption, LORETA finds the smoothest possible distribution of sources (8). Finally, brain network connectivity was assessed with EEG magnitude squared coherence (9). The coherence estimate was computed as magnitude squared coherence (MSC) with values ranging between 0 and 1. These values indicate how well-time series × corresponds to time series y at each frequency bin. The MSC is a function of cross-power spectral density $P_{xy}(f)$ normalized by the individual power spectral densities $P_{xx}(f)$ and $P_{yy}(f)$:

$$C_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xy}(f) * P_{yy}(f)}$$

After computing coherence estimates for all pairs of electrodes, the sTMS-induced change in coherence was defined as the difference between post- and pre-coherence estimates.

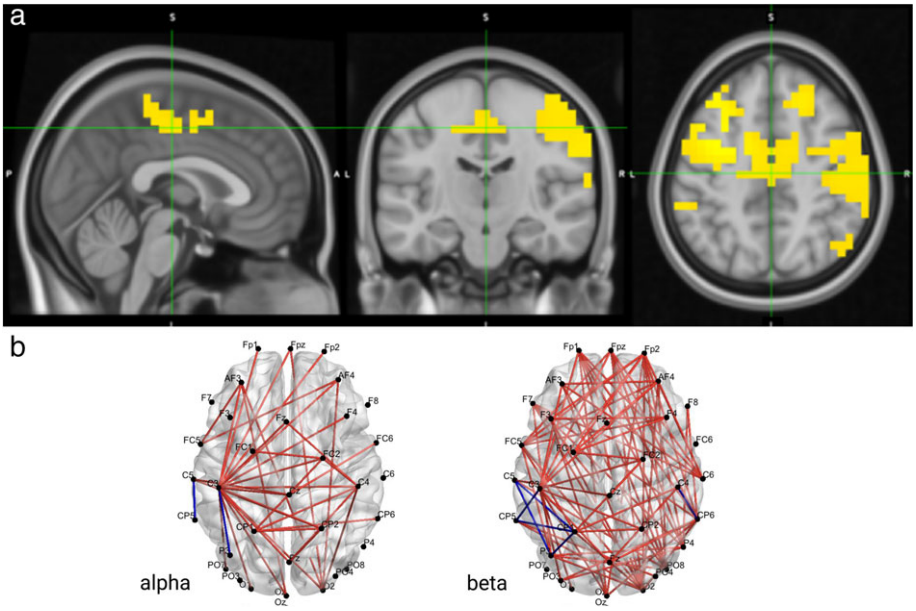

Figure 1. Neurophysiologic findings. a. LORETA map showing voxels with significant correlation of change in current spectral density with contemporaneous symptom improvement in active subjects. b. Coherence maps showing channel pairings with significant correlation at baseline with symptom improvement for active sTMS subjects (red = positive correlation; blue = negative correlation). [Color figure can be viewed at wileyonlinelibrary.com]

In these exploratory analyses with *t*-test, ANOVA, and correlation coefficients, an uncorrected alpha level of 0.05 was employed.

RESULTS

Clinically, active and sham groups did not differ in depression severity at baseline (IDS: Active: 42.8 [8.13 SD] vs. Sham: 44.83 [9.15 SD], $t = 0.46$ $p = 0.65$). Both groups showed clinical improvement (active: 22.00%, SD = 21.76 and sham 48.58%, SD = 25.80), consistent with findings in the primary report for the entire study population (2).

There were no significant differences in absolute or relative power between active and sham groups at any time point in any band, and no significant changes over time within groups. There also were no significant changes in *Q*-factor values, between groups or over-time. There were, however, significant increases in alpha CSD from baseline to final treatment that were positively correlated ($p < 0.05$) with improvement on IDS scores in midline and predominantly anterior voxels in the active sTMS group only (Fig. 1a); no significant correlations were observed in the sham group.

Elevated coherence values in the alpha and beta ranges at pre-treatment baseline were significantly associated with greater IDS improvement at multiple channel pairings, in both inter- and intra-hemispheric connections (Fig. 1b).

DISCUSSION

While this exploratory analysis used a small sample of subjects, our findings can provide useful guidance for hypothesis generation for future research. First, it was notable that classic power measures did not exhibit significant between-group differences or changes over time. Because the size of the recruited network is one determinant of oscillatory power (10), this finding suggests that the size of the neural population generating the alpha rhythm was not expanded through treatment, or that changes in network size were offset by changes in synchrony within the network. There also was no change in the *Q*-factor metric, suggesting that IAF stimulation did not significantly alter the characteristics of the alpha peak.

Second, these analyses provide preliminary evidence of target engagement with the findings using LORETA: 1) CSD increase in the alpha band showed a positive correlation with the degree of clinical improvement; 2) this was found in the active group and not the sham group; and 3) it was observed specifically in voxels near the stimulating magnets, which are placed at anterior and central midline locations. The presence of a source-space CSD change that was correlated with treatment outcome, with the absence of sensor-space power correlations, suggests that clinically-relevant effects may be focal and localized below the cortical surface.

Third, alpha and beta network coherence at baseline was associated with clinical improvement in the active sTMS group. Specifically, beta oscillations have been previously associated with cortical excitability (11,12) and in the present study, the beta band exhibited the largest number of coherence pairings at baseline that were associated with clinical outcome. As a consequence of our findings, we hypothesize that the excitability level of a widespread large-scale network may be predictive of sTMS treatment outcome and recommend this be examined prospectively.

In summary, our findings suggest that there is physiologic engagement of cerebral targets with subthreshold sTMS stimulation, and this may occur focally and deeper than the cortical surface. As well, connectivity measures may represent a potential biomarker to predict successful treatment outcome with sTMS, and merit additional investigation.

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Authorship Statements

Drs. Cook, Corlier, and Leuchter and Mr. Wilson designed and conducted the data analyses. Drs. Cook and Leuchter designed the study, and Dr. Cook recruited the subjects and collected the data. Dr. Cook prepared the manuscript draft with important intellectual input from all coauthors, and all authors approved the manuscript. All authors had complete access to the study data.

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COMMENT

Synchronized TMS offers a novel stimulation paradigm that may be adaptable for home use. This paper offers a concise description of the possible target which determines the efficacy of the intervention.

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Comments not included in the Early View version of this paper.

